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C. Christersson

L. Wallentin

U. Andersson

J. H. Alexander

J. Ansell

Zucker School of Medicine at Hofstra/Northwell

See next page for additional authors

Authors
C. Christersson, L. Wallentin, U. Andersson, J. H. Alexander, J. Ansell, R. De Caterina, B. J. Gersh, C. B. Granger, M. Hanna, A. Siegbahn, and +5 additional authors

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ORIGINAL ARTICLE

D-dimer and risk of thromboembolic and bleeding events in patients with atrial fibrillation – observations from the ARISTOTLE trial

C. CHRISTERSSON,* L. WALLENTIN,*† U. ANDERSSON,† J. H. ALEXANDER,‡ J. ANSELL,§ R. DE CATERINA,¶ B. J. GERSH,** C. B. GRANGER,‡ M. HANNA,†† J. D. HOROWITZ,‡‡ K. HUBER,§§ S. HUSTED,¶¶ E. M. HYLEK,*** R. D. LOPES‡ and A. SIEGBAHN†††††

**Department of Medical Sciences, Cardiology, Uppsala University; †Uppsala Clinical Research Center (UCR), Uppsala University, Uppsala, Sweden; ‡Duke University, Medical Center, Durham, NC; §Lenox Hill Hospital, New York, NY, USA; ¶Gabriele d'Annunzio University, Chieti, and Gabriele Monasterio Foundation, Pisa, Italy; **Mayo Clinic College of Medicine, Rochester, MN; ††Bristol-Myers Squibb, Princeton, NJ, USA; ‡‡University of Adelaide, Adelaide, SA, Australia; §§Department of Internal Medicine, Cardiology and Emergency Medicine, Wilhelminen Hospital, Vienna, Austria; ¶¶Medical Department, Hospital Unit West, Herning/Holstebro, Denmark; ***Boston University Medical Center, Boston, MA, USA; and †††Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden*

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Abstract. *Background:* D-dimer is related to adverse outcomes in arterial and venous thromboembolic diseases. *Objectives:* To evaluate the predictive value of D-dimer level for stroke, other cardiovascular events, and bleeds, in patients with atrial fibrillation (AF) treated with oral anticoagulation with apixaban or warfarin; and to evaluate the relationship between the D-dimer levels at baseline and the treatment effect of apixaban vs. warfarin. *Methods:* In the ARISTOTLE trial, 18 201 patients with AF were randomized to apixaban or warfarin. D-dimer was analyzed in 14 878 patients at randomization. The cohort was separated into two groups; not receiving vitamin K antagonist (VKA) treatment and receiving VKA treatment at randomization. *Results:* Higher D-dimer levels were associated with increased frequencies of stroke or systemic embolism (hazard ratio [HR] [Q4 vs. Q1] 1.72, 95% confidence interval [CI] 1.14–2.59, $P = 0.003$), death (HR [Q4 vs. Q1] 4.04, 95% CI 3.06–5.33) and major bleeding (HR [Q4 vs. Q1] 2.47, 95% CI 1.77–3.45, $P < 0.0001$) in the no-VKA group. Similar results were obtained in the on-VKA group. Adding D-dimer level to the $CHADS_2$ score improved the *C*-index from 0.646 to 0.655 for stroke or systemic embolism, and from 0.598 to 0.662 for death, in the no-VKA group. D-dimer level improved the HAS-BLED score for prediction of major bleeds, with an increase in the *C*-index from 0.610 to 0.641. There were no significant interactions between efficacy and safety of study treatment and D-dimer level. *Conclusion:* In anticoagulated patients with AF, the level of D-dimer is related to the risk of stroke, death, and bleeding, and adds to the predictive value of clinical risk scores. The benefits of apixaban were consistent, regardless of the baseline D-dimer level.

Keywords: apixaban; atrial fibrillation; D-dimer; risk assessment; warfarin.

Correspondence: Christina Christersson, Department of Medical Sciences, Cardiology, Uppsala University, Akademiska Sjukhuset, SE-751 85 Uppsala, Sweden.
Tel.: +46 18 611 90 68; fax: +46 18 50 66 38.
E-mail: christina.christersson@medsci.uu.se

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Introduction

Atrial fibrillation (AF) is associated with an increased risk of stroke and systemic embolism (SEE) and death [1,2]. Oral anticoagulant treatment with vitamin K antagonist (VKA) reduces the risk of stroke more effectively than antiplatelet therapy, but less effectively than some of the newer oral anticoagulants [3–5]. The currently established tools for balancing the benefits of stroke prevention and the bleeding risk during anticoagulant treatment are based on clinical risk scores such as $CHADS_2$/CHA_2DS_2-VASc and HAS-BLED [6–10]. As most clinical risk factors have similar relationships to both thromboembolic and bleeding events, and reflect vascular disease risk factors as opposed to thrombus in the left atrial appendage, the tailoring of treatment to an individual patient is

challenging, and might be improved by the addition of information from biomarkers [11–13]. D-dimer is formed upon degradation of fibrin, and reflects thrombin generation and fibrin turnover [14]. The D-dimer level is a clinically useful tool for the diagnosis of venous thromboembolism, is a risk marker for recurrences after cessation of anticoagulant treatment, and is associated with thrombotic complications in patients with cancer [15–17]. D-dimer levels are increased in patients with AF, and high levels are associated with left atrial appendage thrombus [18–20]. Warfarin treatment reduces D-dimer levels, but the relationship between quality of control of warfarin treatment (i.e. International Normalized Ratio [INR] levels) and D-dimer level has varied in different studies [21–23]. Apixaban, an oral direct factor Xa inhibitor, has been found to reduce D-dimer levels in patients with venous thromboembolism and acute coronary syndrome [24,25]. In the ARISTOTLE trial on patients with AF and at increased risk of stroke, anticoagulant treatment with apixaban as compared with warfarin reduced the rates of stroke and SEE, all-cause mortality, and bleeding [5]. In this study, we evaluated D-dimer level as an independent risk factor for stroke and other non-fatal and fatal cardiovascular events and for bleeding. We also assessed the effects of randomized treatment on outcomes in relation to D-dimer level at baseline.

Patients and methods

The ARISTOTLE trial

The details of the ARISTOTLE trial have been published previously [5,9]. Briefly, ARISTOTLE was a double-blind, double-dummy, randomized clinical trial that enrolled patients with AF and at least one $CHADS_2$ risk factor for stroke or SEE. Patients were randomly assigned (1 : 1) to receive either warfarin (target international normalized ratio [INR] of 2.0–3.0) or apixaban (5 mg twice daily). The number of patients included in the trial was 18 201, and the median follow-up was 1.8 years. The primary endpoint was stroke or SEE, and the primary safety outcome was major bleeds according to the classification of the ISTH [26].

Clinical endpoints and risk factor classification

The endpoints included stroke, defined as a neurologic deficit that lasted for at least 24 h and subclassified as ischemic (with or without hemorrhagic conversion), hemorrhagic, or uncertain, death from all causes, cardiac death excluding fatalities caused by bleeding and non-cardiac causes, and myocardial infarction (MI). Major bleeding was defined as clinically overt bleeding with a decrease in hemoglobin level of at least 2 g L^{-1} or transfusion of two or more units of packed red cells, occurring at a critical site, or resulting in death. Clinically relevant non-major bleeding was defined as clinically overt bleeding that did not fulfill the criteria for major bleeding but that led to hospital admission, physician-guided treatment, or a change in the oral antithrombotic treatment. All clinical events were classified by a blinded clinical event committee using prespecified criteria [27]. $CHADS_2$, CHA_2DS_2-VASc and HAS-BLED scores were calculated individually for each patient on the basis of the sum of risk factors present at randomization. Patients were categorized by $CHADS_2$ according to scores of 0–1, 2, and ≥ 3, by CHA_2DS_2-VASc according to scores of 0–1, 2, 3, 4, and ≥ 5, and by HAS-BLED according to scores of 0–1, 2, and ≥ 3.

Biochemical analysis

At randomization, blood samples for core laboratory analyses of prespecified biomarkers were obtained from 14 878 patients. After centrifugation at 2000 $\times g$ for 15 min, platelet-poor plasma was stored at -80 °C until analysis. D-dimer was centrally analyzed with a quantitative method; ELISA (Asserachrome; Stago, Asnières-sur-Seine, France) in the Uppsala Clinical Research Center (UCR) laboratory, Sweden. The reference limit of normal was ≤ 500 μg L^{-1}. The intra-assay coefficient of variance was 11%.

Statistical analyses

The analyses included the 14 878 patients with available D-dimer results at baseline before randomization to study treatment. The median follow-up time was 1.9 years in the D-dimer cohort. Anticoagulant treatment influences coagulation activity and D-dimer levels [19]. Therefore, the patient cohort was stratified into two groups: no VKA, defined as not treated with oral anticoagulant agents within the last week prior to randomization or study entry ($n = 6867$); and on VKA, defined as treated with oral anticoagulant treatment within the last 7 days ($n = 7982$). In 29 of 14 878 patients, there was no information regarding the last dose of anticoagulant treatment, and these patients were therefore excluded from the analyses. D-dimer levels were compared between VKA groups by use of the Wilcoxon signed rank test. Demographics and other baseline characteristics were summarized by the use of frequencies for categorical variables and median and 25th and 75th percentiles for continuous variables. For tests among groups, the chi-square test was used for categorical variables and the Kruskal–Wallis test was used for continuous variables.

Efficacy analyses included all randomized patients and all events from randomization until the efficacy cut-off date (predefined as 30 January 2011). Bleeding analyses were 'on treatment', including all randomized patients who received at least one dose of study drug, and included all events from receipt of the study drug until 2 days after the

last dose of the study drug. The incidence rates of the different endpoints were summarized in relation to VKA treatment, quartiles of D-dimer levels, and $CHADS_2$, CHA_2DS_2-VASc or HAS-BLED scores, as outlined above. The relationships between D-dimer levels and outcomes were evaluated both in simple and in adjusted Cox regression analysis. The adjusted analyses included randomized treatment, and, for efficacy outcomes, the three different models included $CHADS_2$ score, CHA_2DS_2-VASc score or the variables in the score: congestive heart failure, hypertension, age, diabetes mellitus, previous stroke or transient ischemic attack, previous MI, peripheral arterial occlusive disease, and female sex for efficacy endpoints. For bleeding outcomes, the adjusted analyses included randomized treatment, HAS-BLED score or variables in the score: hypertension, age, previous stroke, previous major bleeding and medication predisposing to bleeding for safety endpoints. The hazard ratios (HRs) and 95% confidence intervals (CIs), with the group with the lowest biomarker levels as reference, were reported. Event rates were reported per 100 patient-years of follow-up. Kaplan–Meier estimates of cumulative hazard were calculated and plotted.

Treatment effects were compared according to D-dimer group with a Cox proportional hazard model including treatment group, $CHADS_2$ or HAS-BLED score, D-dimer quartile group and treatment by D-dimer interaction as covariates. The treatments HRs are reported at each level of D-dimer, regardless of the significance of interaction. Adjustment for CHA_2DS_2-VASc score yielded similar results (data not shown).

The interaction between VKA treatment and D-dimer was investigated with a Cox proportional hazard model, including treatment group, VKA treatment group, D-dimer quartile group and VKA treatment by D-dimer interaction as covariates.

The increased discriminative values of adding D-dimer to models with only $CHADS_2$, CHA_2DS_2-VASc or HAS-BLED score and study treatment were investigated by estimating the *C*-index for the Cox regression models with and without the biomarker [28]. In addition, the continuous (category-free) net reclassification improvement index (NRI) for survival data, as described by Pencina *et al.* [28], was calculated for the total group of 14 849 patients, for the addition of D-dimer to a model with study treatment, baseline VKA treatment and one of $CHADS_2$, CHA_2DS_2-VASc or HAS-BLED score. The NRI among event patients and among non-event patients and the total NRI were analysed. We performed likelihood ratio tests to evaluate whether the global model fit improved after the addition of D-dimer.

All statistical tests were two-tailed and performed at the 0.05 significance level. Owing to the exploratory nature of this study, the *P*-values were not adjusted for multiple comparisons, and should be interpreted with caution. The statistics section at UCR conducted the statistical analyses, using the statistical software package SAS, version 9.3 for Windows (SAS Institute, Cary, NC, USA) for all analyses.

Results

Baseline characteristics in relation to D-dimer levels at baseline

Baseline characteristics are shown in Table 1. The group with no VKA treatment before randomization had a higher proportion of females, a higher rate of left ventricular dysfunction and a lower proportion of diabetes mellitus than the group receiving VKA treatment at randomization. Only 0.78% ($n = 116$) of the patients had a history of cancer. No difference was found regarding previous stroke or $CHADS_2$/CHA_2DS_2-VASc score. The median D-dimer level was 528 μg L^{-1} (interquartile range 335–878 μg L^{-1}) in the total cohort at baseline, and there was no difference in D-dimer levels between the apixaban and warfarin groups. The D-dimer levels in the no-VKA and on-VKA groups are shown in Table 2. Higher D-dimer levels were associated with increased age, female sex, left ventricular dysfunction and atherosclerotic disease in both the no-VKA group and the on-VKA group at randomization (Tables 3 and 4).

D-dimer levels at baseline in relation to thromboembolic events and death

In the D-dimer cohort, a total of 397 patients (1.40% per year) experienced a stroke/SEE, and 1075 (3.69% per year) died; of these, 547 (1.88% per year) died of cardiac causes, and 150 (0.52% per year) suffered an MI. D-dimer levels at baseline were associated with rate of stroke/SEE in both the no-VKA group and the on-VKA group (Fig. 1). In the no-VKA group, the stroke/SEE rate ranged from 1.11% per year in Q1 (D-dimer ≤ 423 μg L^{-1}) to 1.90% per year in Q4 (D-dimer > 1123 μg L^{-1}) (HR [Q4 vs. Q1] 1.72, 95% CI 1.14–2.59, $P = 0.003$ for association with D-dimer levels). Similar results were found in the on-VKA group, with stroke/SEE rates of 0.74% per year in Q1 (D-dimer ≤ 289 μg L^{-1}) and 1.32% per year in Q4 (D-dimer > 690 μg L^{-1}) (HR [Q4 vs. Q1] 1.79, 95% CI 1.13–2.84, $P = 0.027$). After adjustment for $CHADS_2$ score, the association between D-dimer levels and stroke/SEE rate persisted in the no-VKA group but not in the on-VKA group (Fig. 2). Adjustment for CHA_2DS_2-VASc score attenuated the association between D-dimer levels and stroke/SEE rate. Higher D-dimer levels were also related to higher rates of total death (HR [Q4 vs. Q1] 4.04, 95% CI 3.06–5.33, $P < 0.0001$) and cardiac death (HR 2.79, 95% CI 1.97–3.94, $P < 0.0001$) in the no-VKA group. Similar results were obtained in the on-VKA group (total death HR [Q4 vs. Q1] 5.50, 95% CI 4.00–7.57, $P < 0.0001$; cardiac death HR 5.83, 95% CI 3.62–9.39, $P < 0001$). These results persisted after

Table 1 Baseline characteristics in the no-vitamin K antagonist (VKA) and on-VKA groups

	No VKA (n = 6867)	On VKA (n = 7982)	Total (n = 14 878)
Age in years (mean ± SD)	68.5 ± 9.8	69.4 ± 9.3	69.0 ± 9.6
Female sex, no. (%)	2666 (38.8)	2618 (32.8)	5291 (35.6)
Male sex, no. (%)	4201 (61.2)	5364 (67.2)	9587 (64.4)
AF, persistent or permanent, no. (%)	5708 (83.1)	6889 (86.3)	12622 (84.9)
Calculated CrCl (mL min^{-1}), median (Q1–Q3)	72.6 (56.0–92.9)	75.5 (57.5–98.0)	74.2 (56.8–95.4)
Risk factors, no. (%)			
Congestive HF/LVEF ≤ 40%	2727 (39.7)	2619 (32.8)	5353 (36.0)
Age ≥ 75 years	1978 (28.8)	2561 (32.1)	4553 (30.6)
Hypertension	6087 (88.6)	6923 (86.7)	13 033 (87.6)
Diabetes mellitus	1587 (23.1)	2078 (26.0)	3676 (24.7)
Prior stroke or TIA	1253 (18.2)	1531 (19.2)	2788 (18.7)
Prior MI	842 (12.3)	1066 (13.4)	1914 (12.9)
PAOD	320 (4.7)	406 (5.1)	727 (4.9)
$CHADS_2$ score, no. (%)			
0–1	2266 (33.0)	2769 (34.7)	5046 (33.9)
2	2505 (36.5)	2860 (35.8)	5371 (36.1)
≥ 3	2096 (30.5)	2353 (29.5)	4461 (30.0)
CHA_2DS_2-VASc score, no. (%)			
0–1	598 (8.7)	699 (8.8)	1299 (8.7)
2	1435 (20.9)	1658 (20.8)	3099 (20.8)
3	1737 (25.3)	2122 (26.6)	3863 (26.0)
4	1528 (22.3)	1758 (22.0)	3294 (22.1)
≥ 5	1569 (22.8)	1745 (21.9)	3323 (22.3)
HAS-BLED score, no. (%)			
0–1	3150 (45.9)	2351 (29.5)	5503 (37.0)
2	2507 (36.5)	3070 (38.5)	5590 (37.6)
≥ 3	1210 (17.6)	2561 (32.1)	3785 (25.4)
Medical treatment at baseline, no. (%)			
Subcutaneous/intravenous heparin within the last week*	454 (6.6)	269 (3.4)	723 (4.9)
Aspirin	3622 (44.0)	1566 (19.6)	4599 (30.9)
Clopidogrel	160 (2.3)	102 (1.3)	262 (1.8)
ACE inhibitor or ARB	2014 (29.3)	2313 (29.0)	4337 (29.2)
β-Blocking agent	4145 (60.4)	5248 (65.7)	9415 (63.3)
Calcium channel blocking agent	1931 (28.1)	2599 (32.6)	4539 (30.5)
Amiodarone	948 (13.8)	763 (9.6)	1712 (11.5)
Digoxin	2165 (31.5)	2654 (33.2)	4830 (32.5)

ACE, angiotensin-converting enzyme; AF, atrial fibrillation; ARB, angiotensin II receptor blocker; CrCl, creatinine clearance; HF/LVEF, heart failure/left ventricular ejection fraction; MI, myocardial infarction; PAOD, peripheral arterial occlusive disease; SD, standard deviation; TIA, transient ischemic attack. *At least one dose of heparin within the last week.

Table 2 Description of the D-dimer levels at baseline in the no-vitamin K antagonist (VKA) and on-VKA groups

D-dimer	No-VKA group (n = 6867)	On-VKA group (n = 7982)	P-value
Mean (μg L^{-1}) (SD)	1053 (1193)	649 (806)	< 0.0001
Median (μg L^{-1}) (interquartile range)	665 (423–1123)	432 (289–690)	< 0.0001
10th–90th percentiles (μg L^{-1})	299–2097	206–1149	
Range (μg L^{-1})	54–7000	6–7000	
D-dimer > 500 μg L^{-1} (%)	66	42	

SD, standard deviation.

adjustment for $CHADS_2$ score and study treatment in both the no-VKA group and the on-VKA group (Fig. 2), and also after adjustment for CHA_2DS_2-VASc score or the variables included in the score (data not shown). The MI rate was low, and no association was found with D-dimer levels in the no-VKA group. In the on-VKA group, the MI rate ranged from 0.35% per year in Q1 to 1.00% per year in Q4 (HR [Q4 vs. Q1] 2.50, 95% CI 1.34–4.68, P = 0.002 after adjustment for $CHADS_2$ score and treatment) (Fig. 2). These results persisted after adjustment for CHA_2DS_2-VASc score or the variables included in the score (data not shown).

Table 3 Baseline characteristics according to D-dimer quartiles in the no-vitamin K antagonist group

	D-dimer (μg L^{-1})				
	≤ 423 (n = 1719)	> 423–665 (n = 1718)	> 665–1123 (n = 1717)	> 1123 (n = 1713)	P-value
Age in years (mean ± SD)	62.9 (9.54)	67.7 (8.91)	70.5 (8.80)	72.8 (9.16)	< 0.0001
Female sex, no. (%)	480 (27.9)	673 (39.2)	743 (43.3)	770 (45.0)	< 0.0001
Male sex, no. (%)	1239 (72.1)	1045 (60.8)	974 (56.7)	943 (55.0)	
AF, persistent or permanent, no. (%)	1393 (81.0)	1418 (82.6)	1426 (83.1)	1471 (85.9)	< 0.0001
Risk factors, no. (%)					
Congestive HF/LVEF ≤ 40%	635 (36.9)	679 (39.5)	681 (39.7)	732 (42.7)	0.0071
Age ≥ 75 years	180 (10.5)	397 (23.1)	594 (34.6)	807 (47.1)	< 0.0001
Hypertension	1527 (88.8)	1538 (89.5)	1522 (88.6)	1500 (87.6)	0.34
Diabetes mellitus	396 (23.0)	426 (24.8)	406 (23.6)	359 (21.0)	0.0576
Prior stroke or TIA	288 (16.8)	307 (17.9)	307 (17.9)	351 (20.5)	0.0341
Prior MI	173 (10.1)	209 (12.2)	233 (13.6)	227 (13.3)	< 0.0001
PAOD	49 (2.9)	71 (4.1)	76 (4.4)	124 (7.2)	< 0.0001
$CHADS_2$ score, no. (%)					
0–1	755 (43.9)	574 (33.4)	536 (31.2)	401 (23.4)	< 0.0001
2	560 (32.6)	648 (37.7)	632 (36.8)	665 (38.8)	
≥ 3	404 (23.5)	496 (28.9)	549 (32.0)	647 (37.8)	
CHA_2DS_2-VASc score, no. (%)					
0–1	302 (17.6)	138 (8.0)	95 (5.5)	63 (3.7)	< 0.0001
2	530 (30.8)	363 (21.1)	312 (18.2)	230 (13.4)	
3	384 (22.3)	490 (28.5)	463 (27.0)	400 (23.4)	
4	271 (15.8)	370 (21.5)	406 (23.6)	481 (28.1)	
≥ 5	232 (13.5)	357 (20.8)	441 (25.7)	539 (31.5)	
HAS-BLED score, no. (%)					
0–1	987 (57.4)	787 (45.8)	728 (42.4)	648 (37.8)	< 0.0001
2	520 (30.3)	655 (38.1)	650 (37.9)	682 (39.8)	
≥ 3	212 (12.3)	276 (16.1)	339 (19.7)	383 (22.4)	

AF, atrial fibrillation; HF/LVEF, heart failure/left ventricular ejection fraction; MI, myocardial infarction; PAOD, peripheral arterial occlusive disease; SD, standard deviation; TIA, transient ischemic attack. P-value by chi-square test for categorical variables and Kruskal–Wallis test for continuous variables.

D-dimer levels in relation to $CHADS_2$/CHA_2DS_2-VASc score for risk stratification of thromboembolic events and death

The annual rates of stroke/SEE and death increased both with $CHADS_2$ score and with D-dimer levels in the no-VKA group, and a similar trend was found in the on-VKA group. The *C*-index values for D-dimer level alone were 0.587 and 0.567 in the no-VKA group and the on-VKA group, respectively (Table S1). After addition of D-dimer level to the $CHADS_2$ score, the *C*-index increased from 0.600 to 0.618 in the on-VKA group. The *C*-index was higher in the no-VKA group, where the event rate was higher, but the improvement with addition of D-dimer level was somewhat smaller, the *C*-index increasing from 0.646 to 0.655. The model improved significantly only in the no-VKA group. With regard to the prediction of death, the *C*-index values for D-dimer level alone were 0.636 and 0.670 in the no-VKA group and the on-VKA group, respectively. With regard to prediction of cardiovascular death, the *C*-index for D-dimer level was higher in the on-VKA group (0.675) than in the no-VKA group (0.600). The addition of D-dimer level to the $CHADS_2$ score improved the *C*-index most in the on-VKA group, from 0.588 to 0.685, but also in the no-VKA group, from 0.598 to 0.662. When D-dimer level was added to the CHA_2DS_2-VASc score, a similar improvement in the prediction of stroke/SEE and death was noted. The NRI was 11% for stroke/SEE: 18% among event patients, and − 7% among non-event patients. The NRI was 46% for death: 24% among event patients, and 22% among non-event patients (Table S1).

D-dimer levels in relation to efficacy of randomized treatment for thromboembolic events

The benefits of apixaban as compared with warfarin regarding stroke/SEE were similar through D-dimer quartiles, both in the no-VKA group and in the on-VKA group. Accordingly, higher D-dimer levels tended to be associated with a larger absolute gain in stroke/SEE benefit with apixaban than with warfarin, even after adjustments for $CHADS_2$ score (Fig. 3). Also the benefits with apixaban compared to warfarin regarding total death and cardiac death were similar over the range of D-dimer levels (Fig. 3).

D-dimer levels at baseline and bleeding outcomes

Major bleeds occurred in 647 patients (2.61% per year) and 1276 patients had a major/clinically relevant non-major

Table 4 Baseline characteristics according to D-dimer quartiles in the on-vitamin K antagonist group

	D-dimer (μg L^{-1})				
	≤ 289 (n = 2004)	> 289–432 (n = 1990)	> 432–690 (n = 1995)	> 690 (n = 1993)	P-value
Age in years (mean ± SD)	64.0 (9.13)	68.8 (8.54)	71.6 (8.18)	73.3 (8.78)	< 0.0001
Female sex, no. (%)	517 (25.8)	650 (32.7)	743 (37.2)	708 (35.5)	< 0.0001
Male sex, no. (%)	1487 (74.2)	1340 (67.3)	1252 (62.8)	1285 (64.5)	
AF, persistent or permanent, no. (%)	1722 (85.9)	1693 (85.1)	1727 (86.6)	1747 (87.7)	0.1109
Risk factors, no. (%)					
Congestive HF/LVEF ≤ 40%	591 (29.5)	604 (30.4)	654 (32.8)	770 (38.6)	< 0.0001
Age ≥ 75 years	252 (12.6)	539 (27.1)	796 (39.9)	974 (48.9)	< 0.0001
Hypertension	1715 (85.6)	1734 (87.1)	1747 (87.6)	1727 (86.7)	0.2813
Diabetes mellitus	515 (25.7)	535 (26.9)	516 (25.9)	512 (25.7)	0.7972
Prior stroke or TIA	344 (17.2)	398 (20.0)	371 (18.6)	418 (21.0)	0.0136
Prior MI	205 (10.2)	261 (13.1)	279 (14.0)	321 (16.1)	< 0.0001
PAOD	62 (3.1)	69 (3.5)	113 (5.7)	162 (8.1)	< 0.0001
$CHADS_2$ score, no. (%)					
0–1	938 (46.8)	738 (37.1)	596 (29.9)	497 (24.9)	< 0.0001
2	654 (32.6)	668 (33.6)	781 (39.1)	757 (38.0)	
≥ 3	412 (20.6)	584 (29.3)	618 (31.0)	739 (37.1)	
CHA_2DS_2-VASc score, no. (%)					
0–1	379 (18.9)	164 (8.2)	86 (4.3)	70 (3.5)	< 0.0001
2	598 (29.8)	418 (21.0)	375 (18.8)	267 (13.4)	
3	505 (25.2)	573 (28.8)	545 (27.3)	499 (25.0)	
4	293 (14.6)	432 (21.7)	470 (23.6)	563 (28.2)	
≥ 5	229 (11.4)	403 (20.3)	519 (26.0)	594 (29.8)	
HAS-BLED score, no. (%)					
0–1	840 (41.9)	587 (29.5)	452 (22.7)	472 (23.7)	< 0.0001
2	715 (35.7)	770 (38.7)	812 (40.7)	773 (38.8)	
≥ 3	449 (22.4)	633 (31.8)	731 (36.6)	748 (37.5)	

AF, atrial fibrillation; HF/LVEF, heart failure/left ventricular ejection fraction; MI, myocardial infarction; PAOD, peripheral arterial occlusive disease; SD, standard deviation; TIA, transient ischemic attack. P-value by chi-square test for categorical variables and Kruskal–Wallis test for continuous variables.

bleed (5.05% per year) during the median follow-up of 1.9 years. Higher D-dimer levels were significantly associated with increased risk of both major and major/clinically relevant non-major bleeds, regardless of VKA treatment status at baseline (Fig. 4). In the no-VKA group, the rate of major bleeds ranged from 1.61% per year in Q1 (D-dimer < 423 μg L^{-1}) to 2.45% per year in Q4 (D-dimer > 1123 μg L^{-1}) (HR [Q4 vs. Q1] 2.17, 95% CI 1.55–3.04, $P < 0.0001$ after adjustment for HAS-BLED score). Similar results were obtained in the on-VKA group (HR [Q4 vs. Q1] 1.97, 95% CI 1.44–2.69, $P < 0.0001$) (Fig. 2). Similar results were also found after adjustment for the variables included in the score (data not shown).

D-dimer levels in relation to HAS-BLED score for risk stratification of bleeding

The annual risk of major bleeds increased with both HAS-BLED score and D-dimer quartiles. In the no-VKA group, the rates of major bleeds were 1.16% per year in the group with HAS-BLED 0–1 and D-dimer Q1, and 4.94% per year in the group with HAS-BLED ≥ 3 and D-dimer Q4. Similar results were obtained in the on-VKA group: 1.09% per year in the group with HAS-BLED 0–1 and D-dimer Q1, and 5.03% per year in the group with HAS-BLED ≥ 3 and D-dimer Q4. The C-index values for D-dimer for major bleeds were 0.621 and 0.587 in the no-VKA group and the on-VKA group, respectively. Adding D-dimer level to the HAS-BLED score improved the predictive model for major bleeds from 0.610 to 0.641 in the no-VKA group and from 0.618 to 0.635 in the on-VKA group. The NRI was 28% for major bleed: 5% among event patients, and 23% among non-event patients (Table S1).

D-dimer levels in relation to efficacy of randomized treatment for bleeding events

There was no significant interaction between D-dimer levels and randomized treatment with respect to bleeding events. Even after adjustment for HAS-BLED score and VKA status at randomization, apixaban treatment was associated with consistent reductions in the rates of major bleeds and major bleeds/clinically relevant non-major bleeds over the range of D-dimer levels. In the no-VKA group, the rates of major bleeds were 3.21% per year in the apixaban-randomized patients and 4.92% per year in the warfarin-randomized patients in Q4 (D-dimer > 1123 μg L^{-1}) (HR 0.66, 95% CI 0.45–0.97). In the on-VKA group, the HR for apixaban vs. warfarin

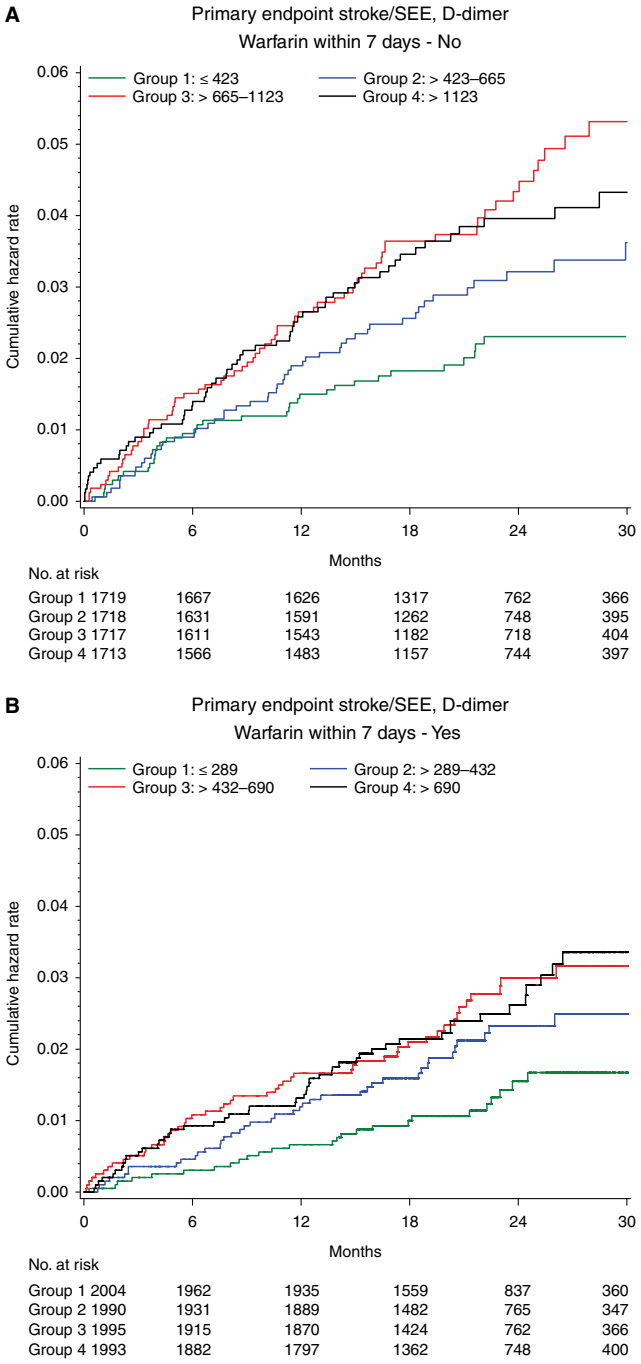

Fig. 1. Cumulative hazard rates for the primary endpoint stroke or systemic embolism (SEE) by D-dimer quartiles at baseline before randomization to apixaban or warfarin study treatment. (A) No-vitamin K antagonist (VKA) group. (B) On-VKA group. Groups 1, 2, 3 and 4 represent D-dimer quartiles 1, 2, 3 and 4, respectively.

was 0.57 (95% CI 0.39–0.82) in D-dimer Q4 (D-dimer > 690 μg L^{-1}) (Fig. 3).

Discussion

This study has verified that D-dimer levels are increased in patients with AF and at least one risk factor for stroke, increase with age, female sex, heart failure, and atherosclerotic diseases. Increasing D-dimer level was gradually related to the rates of stroke/SEE, all-cause death, cardiac death, and major bleeds. Ongoing treatment with warfarin was associated with lower D-dimer levels, but the relationships with patient characteristics were observed both in patients without and those with anticoagulant treatment. The predictive values of D-dimer level alone for death and major bleeds were higher than those of the clinical risk scores. Adding D-dimer to the $CHADS_2$, CHA_2DS_2-VASc and HAS-BLED scores improved the prediction of thromboembolic events and bleeds, respectively. The benefits of apixaban and those of warfarin regarding stroke/SEE, all-cause death, cardiac death and bleeds were not significantly different through the range of D-dimer levels.

D-dimer levels and $CHADS_2$/CHA_2DS_2-VASc scores

In the present study, high D-dimer levels were related to increased risks of stroke/SEE and all-cause death, both total death and cardiac death. In clinical practice, risk stratification of the AF patient is commonly performed with risk scores, such as $CHADS_2$/CHA_2DS_2-VASc, based on clinical features. The $CHADS_2$ score was primarily developed to estimate the risk of stroke in patients without anticoagulant treatment, in order to support decision-making for this therapy [6]. Today, the $CHADS_2$ score has also been found to be predictive of stroke and of death in patients with anticoagulant treatment [10]. In the present study, D-dimer added predictive value to the $CHADS_2$ and CHA_2DS_2-VASc scores for stroke/SEE, and even more value concerning death. D-dimer level alone had an even higher predictive value for death and cardiovascular death than the clinical risk scores, whether or not patients were receiving warfarin treatment. The predictive value was most pronounced in the on-VKA group, indicating that patients with high D-dimer levels despite anticoagulant treatment have a more advanced underlying disease that is not identified by the clinical risk scores. Measurement of the D-dimer level is widely available as a routine method in most hospitals. Therefore, its inclusion in a risk stratification tool for patients with AF might be considered. However, in the real-world environment, there are many different methods for measuring D-dimer levels and fairly large variations in the results, including the influences of preanalytic errors at blood sampling. Therefore, the clinical usefulness of a measurement of D-dimer level is still questionable, even though it is recommended as a tool for risk assessment in the setting of venous thromboembolism [16].

D-dimer and risk of bleeding

Increased D-dimer levels have been associated with bleeding and mortality risk in patients with intracerebral hemorrhage and cancer [29–31]. The D-dimer level reflects the

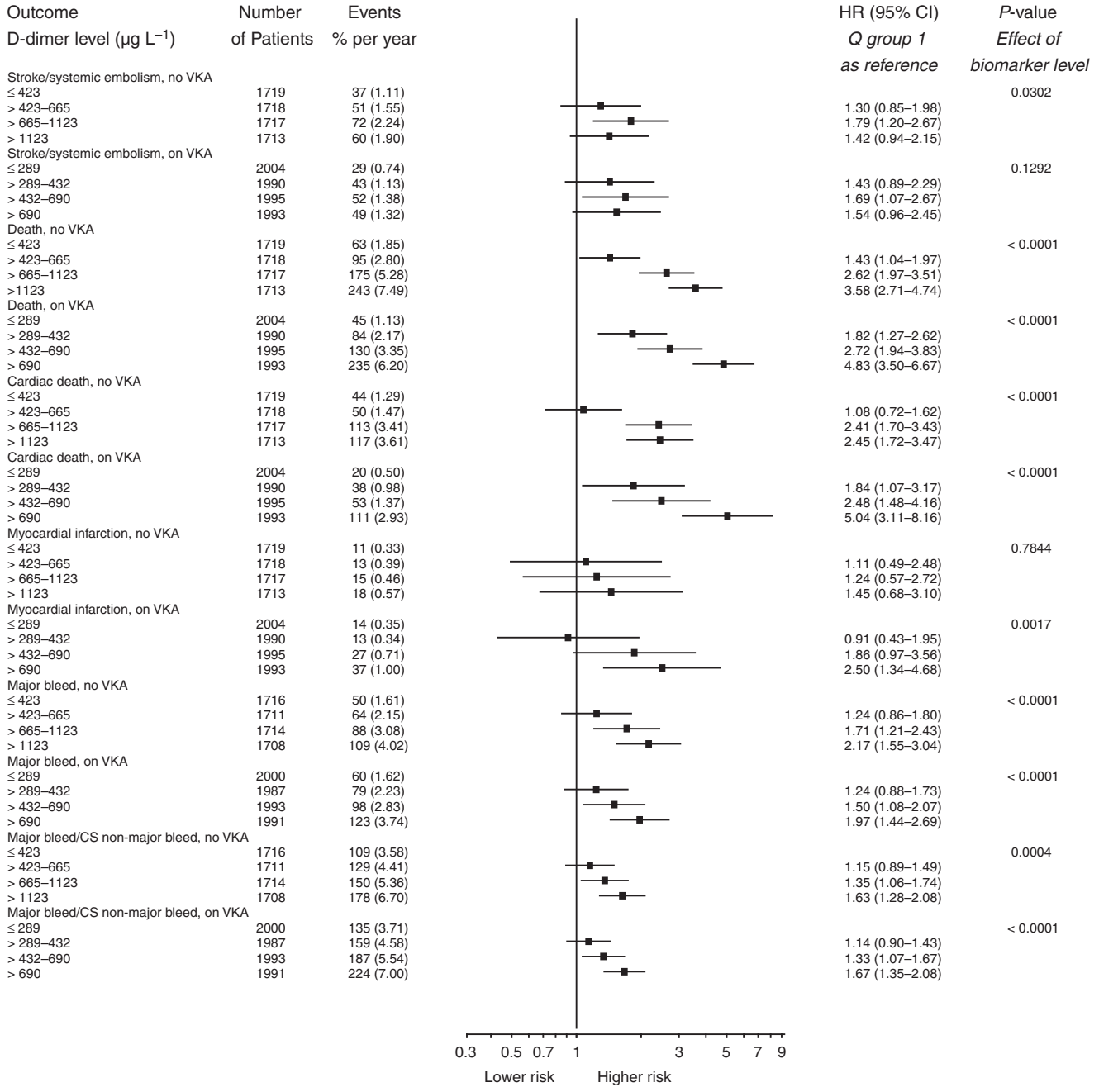

Fig. 2. The effect of D-dimer quartiles on study endpoints in the no-vitamin K antagonist (VKA) group and the on-VKA group. A Cox proportion model was used, with biomarker level, $CHADS_2$/HAS-BLED score and randomized treatment as covariates. CI, confidence interval; CS, clinically significant; HR, hazard ratio.

sum of thrombin generation and endogenous fibrin turnover, and may be a marker for increased fibrinolytic activity [32,33]. However, in clinical practice the risk of bleeding is estimated from clinical history and patient characteristics, and a perceived increased bleeding risk is often used as a reason to withhold anticoagulant treatment in AF patients [34]. The HAS-BLED score can be evaluated in AF patients both with and without anticoagulant treatment, and predicts the risk of bleeding [8,35]. In the present study, increased D-dimer levels were strongly related to the frequency of major and clinically significant non-major bleeds, and added predictive value to the HAS-BLED score to improve bleeding risk stratification. The predictive value of D-dimer level alone for major bleeds was most pronounced in patients who had no experience of anticoagulant treatment. In a clinical setting, where the bleeding risk has to be evaluated before initiation of anticoagulant treatment, D-dimer seems to

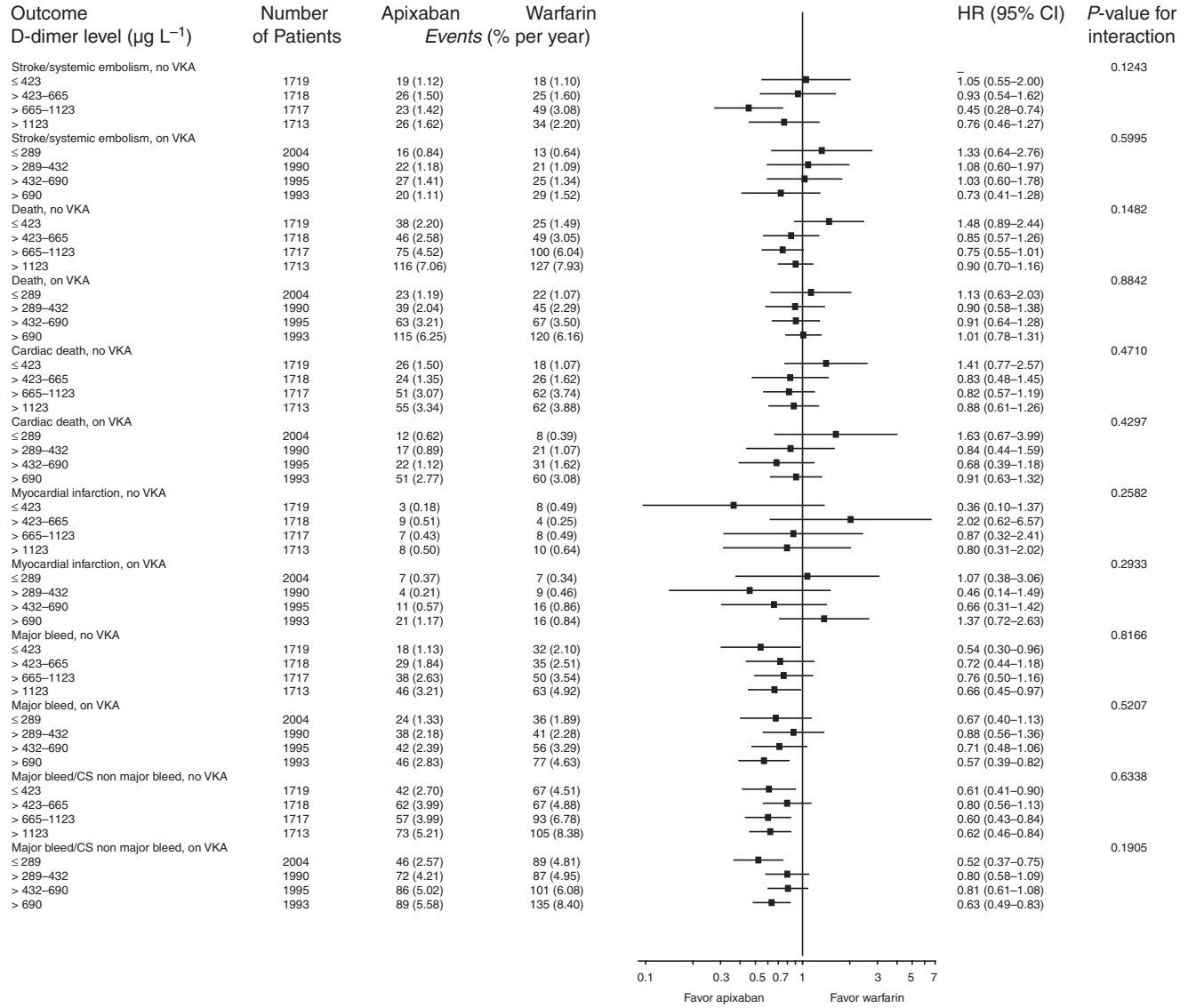

Fig. 3. The efficacy of apixaban vs. warfarin for quartiles of D-dimer levels at baseline separated into the group with no vitamin K antagonist (VKA) treatment and the group receiving VKA treatment before randomization to study treatment. A Cox proportion model was used, with biomarker level, $CHADS_2$/HAS-BLED score, treatment and interaction between treatment and biomarker level as covariates. CI, confidence interval; CS, clinically significant; HR, hazard ratio.

be a better tool than HAS-BLED score, which includes risk factors such as previous experience of warfarin.

D-dimer levels and benefit of apixaban

Apixaban was superior to warfarin regarding the risk of stroke and reduced the frequency of bleeding in the ARISTOTLE trial [5]. The relative benefits of apixaban vs. warfarin have been shown consistently across all $CHADS_2$, CHA_2DS_2-VASc and HAS-BLED scores, even though the relative risk reduction for intracranial bleeding tended to be higher in patients with a HAS-BLED score of ≥ 3 [9]. The relative benefits of apixaban vs. warfarin were not significantly different between D-dimer quartiles.

Limitations

The cut-off of D-dimer level for use in risk stratification in patients with AF has not been defined. In this study, we used the quartiles of D-dimer, and the optimal D-dimer level in AF has to be further investigated and prospectively validated. The INR value in the on-VKA group before randomization and the percent time in therapeutic INR range before randomization to study treatment were not known, and their relationships with D-dimer levels could not be estimated.

Conclusion

In conclusion, a high D-dimer level is associated with increased risks of stroke, death, and bleeding, and has

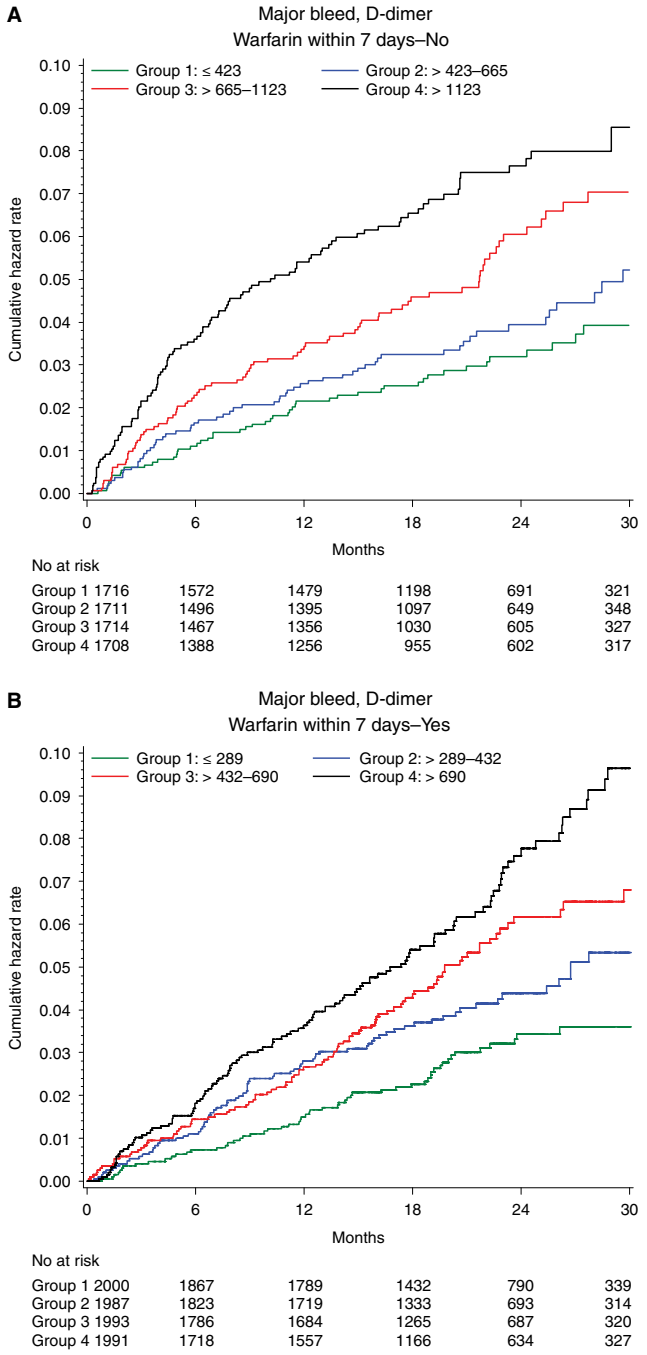

Fig. 4. Cumulative hazard rates of major bleeds in relation to D-dimer quartiles at baseline before randomization to apixaban or warfarin study treatment. (A) No-vitamin K antagonist (VKA) group. (B) On-VKA group. Groups 1, 2, 3 and 4 represent D-dimer quartiles 1, 2, 3 and 4, respectively.

an additional predictive value to clinical risk scores in patients with AF. There was no interaction between the D-dimer level at entry and the benefits of apixaban vs. warfarin for reduction of stroke, mortality, and bleeding.

Addendum

L. Wallentin, J. H. Alexander, C. B. Granger, J. Ansell, B. J. Gersh, M Hanna, J. D. Horowitz, and E. M. Hylek contributed substantially to the design of the ARISTOTLE trial as members of the Executive Committee. R. D. Lopes and E. M. Hylek contributed substantially to the performance of the trial as members of the Clinical Events Committee. C. Christersson, L. Wallentin, J. H. Alexander, C. B. Granger, M. Hanna, J. D. Horowitz, B. J. Gersh, A. Siegbahn, R. D. Lopes, and E. M. Hylek substantially contributed to the design of the ARISTOTLE Biomarker substudy as members of the ARISTOLE Biomarker substudy Committee. C. Christersson, K. Huber, S. Husted, and R. De Caterina contributed to the acquisition of data as ARISTOTLE investigators. A. Siegbahn responsible for the plasma measurements of D-dimer at the UCR laboratory. U. Andersson performed the statistical analyses. C. Christersson and A. Siegbahn provided the first draft of the manuscripts. All authors participated in the interpretation of data, critically revised the manuscript, and approved the final version of the manuscript.

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Disclosure of Conflict of Interests

B. J. Gersh reports receiving personal fees from Medtronic. Inc., Baxter Healthcare Corporation, the Cardiovascular Research Foundation, Merck & Co. Inc., St Jude Medical, Ortho-McNeil Janssen Scientific Affairs, TEVA Pharmaceuticals, and Boston Scientific, outside the submitted work. C. B. Granger reports receiving grants and personal fees from Bristol Myers Squibb, Pfizer, Daiichi, and Boehringer Ingelheim, as well as personal fees from Janssen, during the conduct of the study. C. B. Granger also reports receiving grants and personal fees from Glaxo SmithKline, Sanofi-Aventis, Takeda, and The Medicine's Company; personal fees from Hoffmann-La Roche, Lilly, Ross Medical Corporation, Salix Pharmaceuticals, and Astra Zeneca; and grants from the Medtronic Foundation and Merck & Co., outside the submitted work. E. M. Hylek reports receiving personal fees from Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi Sankyo, Janssen, Pfizer, and Roche, outside the submitted work. J. Ansell reports receiving personal fees from Bristol Myers Squibb, Pfizer, Janssen, Boehringer Ingelheim, and Daiichi Sankyo, outside the submitted work. J. H. Alexander reports receiving personal fees and grants from Bristol-Myers Squibb and Pfizer, during the conduct of the study,

and personal fees from Boehringer Ingelheim, Bayer, and Ortho-McNeil-Janssen, outside the submitted work. L. Wallentin reports receiving grants and personal fees from Bristol-Myers Squibb and Pfizer, during the conduct of the study. L. Wallentin also reports receiving grants and personal fees from AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, and Merck & Co., and personal fees from Abbott, Athera Biotechnologies, and Regado Biosciences, outside the submitted work. M. Hanna is an Executive Medical Director at Bristol-Myers Squibb (BMS), the sponsor of the work described in the present article. Additionally, M. Hanna is the Medical Lead for the apixaban development program, and was the atrial fibrillation lead and sponsor's study director/central medical monitor for the ARISTOTLE study, the trial that generated the data in the present article. Salary compensation is received for his position from Bristol-Myers Squibb. R. De Caterina reports receiving grants and personal fees from Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb, and Daiichi-Sankyo, and personal fees from Roche, outside the submitted work. R. D. Lopes reports receiving grants and personal fees from Bristol-Myers Squibb, and personal fees from Pfizer and Boehringer Ingleheim, during the conduct of the study. R. D. Lopes also reports receiving grants and personal fees from Bristol-Myers Squibb; grants from Glaxo Smith Kline; and personal fees from Bayer, Boehringer Ingleheim, and Pfizer, outside the submitted work. S. Husted reports receiving grants and personal fees from Pfizer and Bristol-Myers Squibb, during the conduct of the study. S. Husted also reports receiving grants and personal fees from Bayer, Boehringer-Ingelheim, and AstraZeneca, and grants from GlaxoSmithKline, outside the submitted work. U. Andersson reports receiving grants from UCR, during the conduct of the study. A. Siegbahn reports receiving grants from Bristol-Myers Squibb, AstraZeneca, and Boehringer-Ingelheim, outside the submitted work. The other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The discriminative value of D-dimer levels as compared with risk scores.

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